

WHAT IS CLAIMED:

1. A nucleic acid molecule comprising a low homology packaging signal cassette flanked by a recombinase recognition sequence, wherein said packaging signal cassette comprises a modified adenovirus packaging signal, provided that said modified packaging signal has low homology to a wild-type adenovirus packaging signal.

2. The nucleic acid of claim 1, wherein said recombinase recognition sequence is *loxP*.

3. The nucleic acid of claim 1, wherein said recombinase recognition sequence is *frt*.

4. The nucleic acid of any one of claims 1-3, wherein said modified packaging signal is less efficient than said wild-type packaging signal.

5. The nucleic acid of claim 4, wherein said wild-type packaging signal is human adenovirus serotype 5 packaging signal.

6. The nucleic acid of claims 5, wherein the modified packaging signal comprises at a maximum, 23 bp of contiguous sequence homology with said wild-type packaging signal.

7. The nucleic acid of claim 5, wherein said modified packaging signal is about 2-3 times less efficient than said wild-type signal.

8. The nucleic acid of claim 6, wherein said modified packaging signal comprises two to six A elements, each A element having a consensus sequence of ATTTGN₈GC.

9. The nucleic acid of claim 6, wherein said nucleic acid is a plasmid.

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10. The nucleic acid of claim 6, wherein said nucleic acid is a helper virus.

11. The nucleic acid of claim 10, wherein said helper virus does not contain an E1 gene.

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12. The nucleic acid of claim 11, wherein said helper virus comprises an E3 region with an insert of about 2.9 kb.

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13. The nucleic acid of claim 12, wherein said insert does not contain a promoter sequence.

14. A nucleic acid comprising an adenovirus E3 gene having an insertion of at least about 2.7 kb, provided that said insertion does not contain a promoter sequence.

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15. The nucleic acid of claim 14, wherein said insertion is a human intron sequence.

16. An adenoviral helper virus for production of helper dependent vectors comprising:

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(a) an adenovirus genome having an E1 region deletion;

(b) an excisable packaging signal cassette replacing a wild-type packaging signal, the excisable packaging signal cassette comprising a 5' *loxP* site, a modified packaging signal and a 3' *loxP* site, wherein said modified packaging signal has low homology to and is less efficient than the wild-type packaging signal; and

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(c) an optional insertion element comprising at least about 2900 base pairs of non-adenoviral DNA inserted in the E3 region without deleting any part of the E3 region.

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17. The virus of claim 16 wherein said adenovirus genome is human Adenovirus type 5.

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18. The virus of claim 17 wherein said modified packaging signal comprises at a maximum, 23 bp of contiguous sequence homology with said wild-type packaging signal.

5 19. The virus of claim 18, wherein said modified packaging signal is about 2-3 times less efficient than said wild-type signal.

10 20. The virus of claim 19, wherein said modified packaging signal comprises two to six A elements, each A element having a consensus sequence of ATTTGN_gGC.

15 21. A vector comprising the adenovirus of claim 16.

22. A cell line expressing E1 and infected with the helper virus of claim 16.

20 23. The cell line of claim 22, wherein said cell line further expresses *cre* recombinase.

24. The cell line of claim 23, wherein said cell line is 293 *cre* cells.

25 25. A helper-dependent adenovirus vector comprising:
a) a 5' ITR;
b) a packaging signal;
c) at least one heterologous expression cassette;
d) a human genomic stuffer DNA;
e) an optional E4 non-coding segment conferring a selective advantage, wherein the E4 element is located between nucleotide -400 from the right end; and

30 f) a 3' ITR;
wherein the overall size of the vector is between about 28 kb and 36 kb, and wherein the only adenoviral sequences present are the ITRs, optional E4 non-coding segment and the packaging signal, and wherein no bacterial origin of replication or bacterial marker genes are present.

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26. The helper dependent adenovirus vector of claim 25, wherein said optional E4 element is present.

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27. A plasmid vector comprising

- a) a 5' ITR;
- b) a packaging signal;
- c) at least one heterologous expression cassette;
- d) a human genomic stuffer DNA;
- e) an optional E4 non-coding segment conferring a selective advantage, wherein the E4 element is located between nucleotide -400 from the right end; and
- f) a 3' ITR.

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28. A helper-dependent adenovirus comprising in a 5' to 3' direction:

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- a) a 5' ITR,
- b) a packaging signal cassette directly joined to the 3' of said 5' ITR,
- c) a first stuffer DNA at least about 1 kb,
- d) at least one heterologous expression cassette,
- e) a second stuffer DNA at least about 1 kb,
- f) an optionally present non-coding E4 segment at least 400 bp in length; and

25 g) a 3' ITR, wherein said 3' ITR is directly joined to the 5' end of said non-coding E4 segment if present;

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provided that said helper-dependent adenoviral vector does not encode one or more proteins needed for viral generation, is about 28 kb to about 36 kb, and has a GC content between about 50% and about 60%.

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29. The helper-dependent adenoviral vector of claim 28, wherein said virus is between 30 and 36 kb in length.

30. The helper-dependent adenoviral vector of claim 29, wherein said first stuffer and said second stuffer are derived from inverted mammalian non-gene or intron sequences.

31. The helper-dependent adenoviral vector of claim 30, wherein said virus does not encode for any adenovirus proteins.

5 32. The helper-dependent adenoviral vector of any one of claims 28-31, wherein said optionally present non-coding E4 region is present.

10 33. The helper dependent virus of claim 32, wherein said GC content is between 52% to 57%.

15 34. A method of generating helper-dependent adenoviral gene vectors in a cell line expressing E1 and *cre* recombinase comprising:

15 a) infecting said cell line with a helper-dependent vector comprising: a 5' ITR, a packaging signal, at least one heterologous expression cassette, human genomic stuffer DNA and a 3' ITR, wherein the overall size of the helper-dependent vector is between about 28 kb and 36 kb, and wherein no functional adenoviral coding sequences and no bacterial origin of replication or bacterial marker genes are present;

20 b) infecting the cell line with a helper virus comprising: an adenovirus genome having an E1 region deletion; an excisable packaging signal cassette replacing a wild-type packaging signal, the excisable packaging signal cassette comprising a 5' *loxP* site, a modified packaging signal and a 3' *loxP* site, wherein the modified packaging signal has low homology to and is less efficient than the wild-type packaging signal; and an optional insertion element comprising at least about 2900 base pairs of non-adenoviral DNA inserted in the E3 region without deleting any part of the E3 region; and

25 c) obtaining the generated helper-dependent viral vectors.

30 35. A method of generating a helper-dependent adenoviral vector comprising:

a) producing a cell comprising

(i) *trans* functions needed for adenovirus generation; and
(ii) said helper-dependent adenoviral vector, wherein said

helper-dependent adenoviral vector comprises the necessary *cis* functions needed for

adenovirus generation and at least one heterologous expression cassette, and said helper-dependent adenoviral vector does not encode for any adenovirus proteins, is about 28 kb to about 36 kb, and has a GC content between about 50% and about 60%, and

5 b) generating said helper-dependent adenoviral vector.

36. The method of claim 35, wherein late proteins and either E2 proteins or E4 proteins, or both E2 proteins and E4 proteins are supplied by a helper virus present in said cell.

10 37. The method of claim 36, wherein said helper virus comprises a low homology packaging signal cassette flanked by a recombinase recognition sequence, wherein said packaging cassette signal comprises a modified adenovirus packaging signal having low homology to a wild-type adenovirus packaging signal.

15 38. The method of claim 37, wherein said recombinase recognition sequence is *loxP* and said cell expresses Cre recombinase.

20 39. The nucleic acid of claim 37, wherein said recombinase recognition sequence is *frt* and said cell expresses Flp recombinase.

40. The method of claim 37, wherein said modified packaging signal is less efficient than said wild-type signal.

25 41. The method of any one of claims 36-40, wherein said wild-type packaging signal is from human adenovirus serotype 5.